EFFECTS OF ANABOLIC CYTOKINES AND STATIC COMPRESSION ON MENISCUS TISSUE EXPLANTS

Stacy M. Imler (1,2), Ashish N. Doshi (2), Marc E. Levenston (1,2)

(1) Woodruff School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA

(2) Georgia Tech/Emory Center for the
Engineering of Living Tissues
Georgia Institute of Technology,
Atlanta, GA

INTRODUCTION

The knee meniscus is a fibrocartilaginous soft tissue critical for maintenance of normal knee biomechanics. Injury to this soft tissue affects a sizeable fraction of the population, with the incidence of meniscal tears reported as 0.7 per 1000 inhabitants [1]. Autonomous repair in response to injury is limited due to the avascular nature of the tissue. However, it is known that cytokines play an essential role in the development, maintenance, and repair of soft tissues in the knee. The meniscal cells, fibrochondrocytes, rely on diffusion of these factors and other nutrients from the joint space and through the tissue matrix. Recognizing specific anabolic cytokines as major stimulators of cartilaginous extracellular matrix growth, it is important to characterize their actions and potency on fibrochondrocytes in tissue explant culture. In addition, the response to physiologic mechanical stimuli may be modified by the action of specific cytokines. The goals of this study were a) to investigate the dose- and time-dependent stimulation of meniscal explant protein and proteoglycan production for four anabolic cytokines and b) to investigate the response to static compression when cultured with each of these cytokines.

METHODS

Full thickness meniscal cores (4mm diam) were obtained from both menisci of immature (2-4 week) bovine stifle joints and trimmed to a prescribed thickness. Meniscal explants were precultured in basal medium (DMEM + 0.1% BSA, NEAA, 50 μg/ml ascorbate, and 0.4 mM proline) for 3 days. In all experiments, media were supplemented with 20 μCi/ml L-5-^{3}H-proline and 10 μCi/ml ^{35}S-sodium sulfate for the final 21 hours of the culture period. **Dose-response** Meniscal explants (1 mm thick, n=12-18/condition) were cultured in basal medium and various levels of the following anabolic cytokines for 4 days (Table 1): basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor-AB (PDGF-AB), or transforming growth factor-β1 (TGF-β1). **Time-course** Meniscal explants (1 mm thick, n=5/condition/time point) were cultured in basal medium unsupplemented or supplemented with a single level of each cytokine based on saturation or maximal levels from the dose-responses (Table 1). Explants were cultured for 2, 4, 7, or 14 days. **Static compression** Meniscal explants (2 mm thick, n=6 per condition) were cultured in basal medium unsupplemented or supplemented with a single level of cytokine (Table 1) under static compression to 0%, 25%, or 50% of the original 2mm cut thickness. Additional free-swelling (FS) groups were maintained uncompressed in 48-well plates. Explants were cultured for 4 days. **Analysis** Samples were lyophilized, digested in proteinase K, and assayed for DNA, ^{3}H, and ^{35}S content. Conditioned media were assayed for sGAG and nitrite content. Data were analyzed using multifactor General Linear Models ($p<0.05$) and Tukey's test for post-hoc analyses. Data are presented as incorporation rates per DNA content normalized to the means for BSA controls (growth factor experiments) or FS controls (compression experiments).

	Dose-response			Time-course	Static Compression
	Lower	-	Upper		
TGF-β1	0.05	-	5	5	50
IGF-1	30	-	1000	200	100
PDGF-AB	3	-	300	100	100
bFGF	1	-	300	100	100

Table 1: Cytokine concentrations (ng/ml)

RESULTS

Dose-response For IGF-1 and PDGF-AB, the highest concentrations reached saturated levels of both proline and sulfate incorporation (Fig. 1b, 1c). bFGF did not stimulate proline incorporation at any concentration, but significantly stimulated sulfate incorporation at the highest levels (Fig. 1d). TGF-β1 significantly stimulated both proline and sulfate incorporation, but saturation was not achieved in the range examined (Fig. 1a). For all four cytokines, the relative increase in sulfate incorporation was significantly greater than the increase in proline incorporation. **Time-course** All four cytokines induced significant increases in sulfate incorporation that were sustained over the 14 days of culture (Fig. 2a), with TGF-β1

producing the greatest stimulation. In contrast, little increase in proline incorporation was seen for any cytokine at any point in the 14 day culture period (data not shown). Release of sGAG to the media was consistent with the incorporation data, with the greatest release of sGAG induced by TGF-β1 (Fig. 2b). For the entire study, the levels of nitrite in the media for all groups were undetectable using the Griess reaction. **Static Compression** In the presence of any one cytokine, both normalized proline and sulfate incorporation were significantly inhibited for all static compression percentages as compared to the FS groups (Fig. 3a, 3b). Increasing compression levels induced a significant dose-dependent inhibition of proline incorporation, but not of sulfate incorporation. Interestingly, although the overall levels of synthesis varied substantially between media conditions, the dose dependent inhibition of proline incorporation relative to FS synthesis levels did not vary significantly between media conditions.

DISCUSSION

These data illustrate the potent effects of anabolic cytokines on sulfate and proline incorporation (indicators of proteoglycan and total protein production, respectively) by fibrochondrocytes in meniscal explant culture. All of the chosen factors stimulated matrix synthesis in a dose-dependent manner, with stimulation of proteoglycan synthesis maintained for 14 days. Previous data for meniscal explants using only IGF-1 [2] agree well with the observations of this study with sustained stimulation of proteoglycan production and transient stimulation of protein production over 14 days of culture. The concentration ranges were chosen based upon previous studies in the literature. We anticipated capturing a saturation level of stimulation with the highest concentrations, but neither TGF-β1 nor bFGF appeared to produce saturation in the tested range.

Although the responses of meniscal tissue to these cytokines were generally similar to those previously reported for articular cartilage, the response to bFGF was somewhat different. While anabolic at low concentrations, bFGF at greater than 30 ng/ml has been shown to have a catabolic effect on cartilage explants [3]. In contrast, we observed significant stimulation of proteoglycan synthesis with up to 300 ng/ml of bFGF. Such differences in the behaviors of these two tissues may have implications for the development of cytokine-based repair strategies for either tissue. As the tissues occupy a common synovial environment in the knee, care must be taken to ensure that a treatment targeting one tissue does not have detrimental effects on the other.

The effects of continuous static compression were consistent with those previously reported for meniscal tissue cultured in serum supplemented medium [2]. Interestingly, when normalized to the uncompressed FS group for each media supplement, the relative inhibition of protein synthesis by static compression did not significantly vary between media conditions. This suggests that, at least in terms of the response to static compression, the biochemical and biomechanical stimuli have distinct, non-interacting effects on meniscal protein synthesis. Proteoglycan synthesis was significantly inhibited in all compression groups compared to FS, but this inhibition did not vary with compression level. In previous short-term studies, we did not detect any significant inhibition of meniscal proteoglycan synthesis. In the present study, the free swelling explants swelled considerably over the culture period, suggesting that even the "0% compression" group held at the cut thickness developed a substantial compression relative to free swelling over the four day culture period. These studies begin to elucidate the influences of the biochemical and biomechanical environment on meniscal metabolism, and will contribute to the design and understanding of fibrocartilage repair strategies.

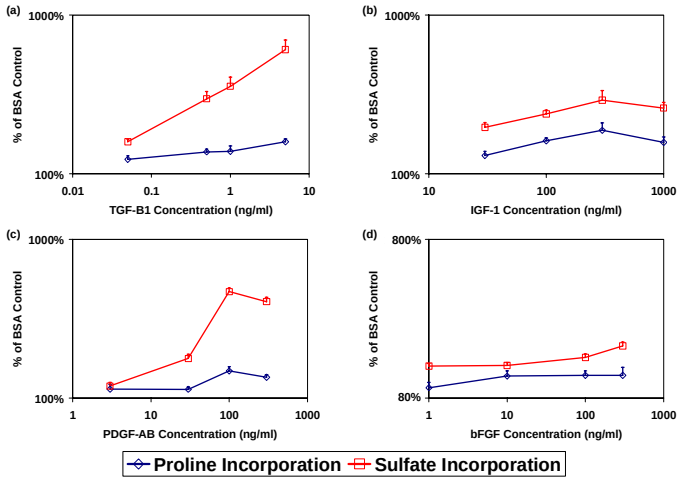


Figure 1. Dose-responses for (a) TGF-β1, (b) IGF-1, (c) PDGF-AB, and (d) bFGF

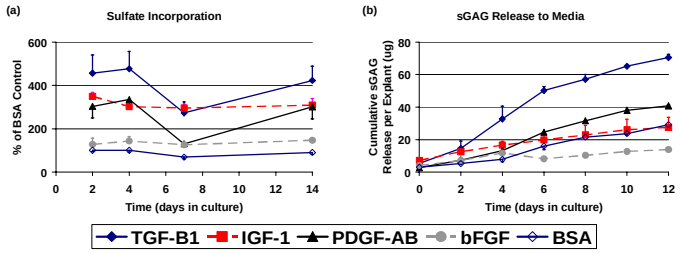
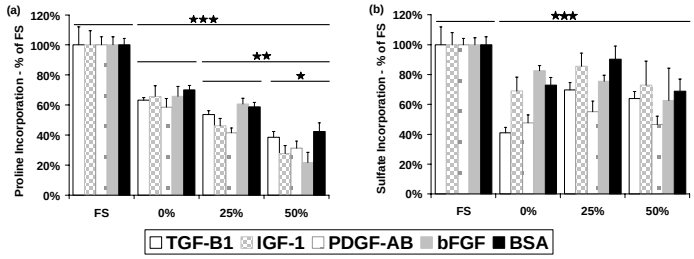


Figure 2. (a) Time-course of proteoglycan synthesis and (b) cumulative sGAG released to media per explant



All figures mean ± s.e.m.

Figure 3. Effects of static compression on (a) proline incorporation and (b) sulfate incorporation

ACKNOWLEDGMENTS

This work was supported by an Arthritis Foundation Arthritis Investigator grant, the ERC and REU programs of the NSF, and by NSF and Luce Foundation Fellowships to SMI.

REFRENCES

1. Hede A, 1993, "Treatment of Meniscal Lesions in the Knee," Danish Medical Bulletin, Vol. 40, pp. 317-331.
2. Imler SM, Vanderploeg EJ, Hunter CJ, Levenston ME, 2001, "Static and Oscillatory Compression Modulate Protein and Proteoglycan Synthesis by Meniscal Fibrochondrocytes," Transactions of the Orthopaedic Research Society, Vol. 48, p. 366.
3. Sah RL, Chen AC, Grodzinsky AJ, Trippel SB, 1994, "Differential Effects of bFGF and IGF-1 on Matrix Metabolism in Calf and Adult Bovine Cartilage Explants," Archives of Biochemistry and Biophysics, Vol. 308, pp. 137-147.